The Mechanism of Ciliary Movement. II.—The Effect of Ions on the Cell Membrane.

By J. Gray, M.A., Fellow of King's College, Cambridge, and Balfour Student in the University of Cambridge.

(Communicated by Prof. J. S. Gardiner, F.R.S. Received October 7, 1921.)

In 1906 R. S. Lillie (7) published an account of the effects of various pure sodium salts upon the ciliated epithelium of *Mytilus edulis*. He found that the ions could be arranged in the following order of "toxicity,"

$$Cl' < NO_3' < Br' < I' < SCN'.$$

Lillie also referred to the effect which various anions have upon the amount of water taken up by the cells from their external medium, and arranged the ions in the following order of efficiency in causing an absorption of water by the cells.

$$CH_3COO' < Cl' < NO_3' < ClO_3' < Br' < I' < SCN' < BrO_3' < OH'.$$

These results form the basis of the following statements found in recent text-books, viz., Höber (6) and Bechhold (1):—

(i) That the above series represents the effect of anions on ciliary movement.

(ii) That the order in which anions affect ciliary movement is the reverse of that in which they affect muscular movement.

One of the objects of the present communication is to consider whether these important statements are justified.

It has already been shown (Gray (3)), that if the ciliated epithelium of *Mytilus edulis* is placed in a solution containing NaCl, KCl, $CaCl_2$ and $MgCl_2$, the whole tissue remains normal and in activity for a very prolonged period. If we wish, therefore, to determine what specific *rôle*, if any, is being played by the chlorine ion, it is necessary to replace this ion in the above solution by other anions. It is not permissible to use a solution which does not contain either $K^{\cdot}$, $Ca^{\cdot\cdot}$, or $Mg^{\cdot\cdot}$, nor is it permissible to ignore the hydrogen ion concentrations of any experimental solution (Gray (3)).

In the experiments tabulated in Table I, each solution contained the same molecular concentration of cations, and the hydrogen ion concentration was kept well within the limits to which the tissue is indifferent.

Table I.—The Effect of Mixtures of $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, and $Mg^{\cdot\cdot}$ upon the Movement of the Terminal Cilia of *Mytilus*.

Anion.	Time.					PH.
	30 mins.	1 hour.	2 hours.	3 hours.	5 hours; exp. discontinued.	
Chlorides	Normal movement; no absorption of water.	Normal movement; no absorption of water.				7·8
Nitrates	Normal movement; no absorption of water					7·8
Iodides	Very rapid movement; no absorption of water.			Normal movement; no absorption of water.		8·0
Bromides	Normal movement; no absorption of water					7·8
Acetates	Normal movement; no absorption of water					7·9
Sulphates	Very rapid movement; no absorption of water.		Normal movement; no absorption of water.			8·0
Tartrates	Normal movement.	Slow movement.	Very slow movement.	No movement		7·8
	No absorption of water.					
Citrates	No movement; no absorption of water					7·8

Such experiments shew quite clearly that in a solution containing balanced cations, the substitution of the normal anion chlorine by NO_3', I', Br', Ac', or SO_4'' is not attended by any interference with either the activity of the cilia or the amount of water taken up by the cells. It is possible that in the case of the iodide and sulphate mixtures there is an actual increase in the rate of beat of the cilia, although the observed effects may possibly be due to the slightly higher hydroxyl ion concentration of these solutions.

In considering the possible significance of the cessation of movement in balanced solutions of tartrates and citrates, we are confronted with a purely chemical problem. Calcium tartrate and calcium citrate are both very sparingly soluble in water; the same statement applies, though to a less degree, to the magnesium salts. When, however, these salts are added to a solution of the corresponding sodium salt, they dissolve to a very marked extent. The suspicion arises that in making up a physiological mixture of tartrates or citrates, the metals magnesium and calcium are not present as free ions. This suspicion seems justified by the following facts. When sodium phosphate and ammonia are added to a saturated solution of magnesium citrate in water, a definite precipitate of magnesium phosphate is formed. If, however, sodium phosphate and ammonia are added to a solution of magnesium citrate in sodium citrate, no precipitate is formed, although a considerable amount of magnesium is in solution. We may conclude, therefore, that the inability of tartrate or citrate mixtures to maintain the normal equilibrium of ciliated cells may prove to be due to the absence of free magnesium and calcium ions and not to the direct effect of the anions. Confirmation of this view will be given later in this paper.

As far as I am aware, the only satisfactory investigation of the effects of anions on other living processes is that of Sakai (9) on the heart of the frog. This author used solutions containing balanced cations, but does not refer to their hydrogen ion concentration. His results may be summarised as follows:—

I', Br', NO_3'	Beat well maintained, even faster than normal.
Cl'	Normal.
SO_4''.........	Beat well maintained, after initial slowing.
Cit'''	Rate steadily falls and finally heart stops.

There is clearly no fundamental difference between the effect of anions on ciliary and on muscular activity. Both types of tissue are remarkably indifferent to wide variations in the nature of the anions in the external environment.

(b) *The Effects of Solutions of Pure Sodium Salts.*

Whereas in a solution of a sodium salt containing $K^{\cdot}$, $Ca^{\cdot\cdot}$, and $Mg^{\cdot\cdot}$, the ciliated epithelium is remarkably indifferent to a variation in the nature of the anions present, yet in a solution of a sodium salt, which does not contain other cations, the tissue shows a very marked sensitivity to particular anions. Table II shows the general course of events.

It will be seen that the salts can be divided into two main groups:

A. Those salts which cause the cells to swell up by the absorption of water. $Cl' <$, $NO_3' <$, $Br' <$, I';

B. Those salts which do not cause the cells to swell. SO_4'', Tartrate, Citrate;

while the acetate forms an intermediary type.

It is also clear that these solutions cannot indicate the direct effect of the environment on the contractile mechanism; their effect on the cell is of a more general nature, and the contractile mechanism is only secondarily involved.*

---

* Cilia often remain active when the process of absorbing water is relatively far advanced.

Table II.—The Effect of Pure Sodium Salt Solutions upon Ciliated Epithelia.

Anion.	Time.					P_H.
	30 mins.	1 hour.	2 hours.	3 hours.	5 hours.	
Chloride…………	Some cilia active. A little swelling.	Cells considerably swollen. Some cilia destroyed.	Tissue disorganised. Cells much swollen. Cilia destroyed.			7·8
Nitrate …………	A few cilia active. Most cells begin to swell.	Cells much swollen. Few cilia left.	Tissue disorganised. Cells much swollen. Cilia destroyed.			7·8
Bromide…………	A few cilia active. Most cells begin to swell.	Cells much swollen. Few cilia left.	Tissue disorganised. Cells much swollen. Cilia destroyed.			7·8
Iodide …………	Nearly all cells markedly swollen.	Tissue disorganised. Cells much swollen. Cilia destroyed.				8·0
Acetate …………	No movement. Cells normal.	No movement. Cells slightly swollen				7·9
Sulphate ………	No movement: cells do not swell. Cilia remain healthy in appearance.					8·0
Tartrate ………	No movement: cells do not swell. Cilia remain healthy in appearance.					7·8
Citrate …………	No movement: cells do not swell. Cilia remain healthy in appearance.					7·8

The effects of the various sodium salts upon the water-content of the cells is precisely what one would expect from the effects of the same salts on the water-content of such non-living colloid gels as fibrin or gelatine. Such gels swell readily in the presence of hydroxyl ions, but the uptake of water is affected by different salts in precisely the same way as we have found for ciliated cells.

Now, during life the interior of the cell is always more acid than the external medium; although at the same time it must be remembered that the cell colloids are on the alkaline side of their isoelectric point. Hence, if the cell interior is allowed to come into contact with the external medium, the amount of water taken up at any particular hydrogen ion concentration will depend upon the nature of the anions present in the medium. This is seen above actually to be the case, when the cells are put into pure solutions of sodium salts. It is not the case when the cells are in a solution containing balanced cations. In the latter type of solution the hydroxyl ions outside the cell cannot penetrate the cell, nor can the anions outside the cell exert any influence upon the uptake of water by the cell. Such a condition of affairs is obviously due to the semi-permeable nature of the normal cell membrane. The inference to be drawn is, of course, that when placed in a solution of a pure sodium salt the observed effects are the direct outcome of the loss of semi-permeability by the cell membrane. Direct proof of this statement is provided by a determination of the electrical conductivity of normal tissues placed in pure sodium salts. Osterhout (8) showed that the electrical resistance of *Laminaria* tissue placed in sodium chloride solution fell steadily, until it was one-third of its original value; Gray (4) showed that sodium citrate caused a marked fall in the resistance of Echinoderm eggs, and quite recently Shearer (11) has shown the similar effects of sodium chloride on bacteria.

(c) *The Nature of the Cell Membrane.*

Lillie (7) showed that the toxic effect of pure sodium salts is prevented by the presence of the alkaline earth metals. This has been confirmed and the statement can be somewhat enlarged. In the first place, either magnesium or calcium can prevent the destructive action of a pure sodium chloride solution upon the cell-membrane; or, as is perhaps the more correct mode of expression, the presence of magnesium or calcium is necessary for the semi-permeable properties of the cell membrane. Since both calcium and magnesium occur in sea-water, it is of importance to determine, if possible, which of these two metals maintains the normal stability of the cell surface.

In the following experiment the concentration of each salt was the concentration in which it normally occurs in sea-water.*

$NaCl$	P_H 7·0	Complete disintegration after 3 hours.
$NaCl + CaCl_2$	P_H 7·0	No disintegration after 24 hours. Some cilia destroyed, and some filaments separated from their neighbours.
$NaCl + MgCl_2$	P_H 7·0	Tissue quite healthy, but cilia motionless after 24 hours.

It is clear that the amount of magnesium in normal sea-water is alone capable of maintaining the cell-surface: at the same time the amount of calcium is also sufficient to produce a very well-marked stabilising influence. In the solution which contains only calcium and sodium in their normal concentrations, the tissue does not remain so healthy as in that containing magnesium and sodium; in the former solution there is a distinct tendency for the finer cilia to become opaque and detached from the cell: this is particularly the case with the small cilia on the ciliated junctions, so that individual gill filaments show a distinct tendency to separate from each other (see Gray (3)). Interesting facts concerning the action of calcium are, however, available from a study of cells whose normal semi-permeability has been destroyed by immersion in those sodium salts which prevent the uptake of an abnormal amount of water by the cell. When ciliary movement has ceased in sodium citrate, the cilia and the tissue appear quite healthy and translucent. On transference to normal sea-water, however, the cilia at once become opaque, and are completely destroyed; at the same time there is a rapid uptake of water by the cells which swell up in the usual way. If, on the other hand, the tissue (after initial citrate treatment) be placed in sea-water containing no calcium, the cells and cilia remain quite healthy, and complete recovery of movement takes place, although the rate of beat is usually slower than the normal; after a time the rate of beat falls off, but can be revived permanently by adding calcium. The same recovery from sodium citrate treatment can be effected by treating the tissue with any solution containing magnesium but no calcium. The same experiments can be performed with tissues previously treated with sodium sulphate or sodium tartrate. It is clear from numerous experiments that magnesium is the only ion in sea-water which will re-form a semi-permeable membrane round a cell which has lost this structure by exposure to a pure sodium salt.

---

* The composition of the sea-water used was :—$NaCl$, 28·3 grs. per litre ; KCl, 0·76 grs. per litre ; $MgCl_2$, 5·01 grs. per litre ; $CaCl_2$, 1·22 grs. per litre.

At this point it is interesting to note that calcium has a double action on ciliated cells: (*a*) It is capable of maintaining the cell surface in a normal state of semi-permeability; (*b*) it is necessary for continuous movement in a solution of P_H 7·0 (see Gray (5)). In the first of these functions calcium can be completely replaced by magnesium, but cannot be so replaced in the second.

The destructive effect of sodium salts upon the cell membrane is shared by other monovalent cations, although to a variable extent. In the case of the chlorides, there is little difference between $Na^{\cdot}$, $NH_4^{\cdot}$, and $K^{\cdot}$, while the effect of $Li^{\cdot}$ is considerably less. In the case of other salts, *e.g.*, tartrates and citrates, the erosive power of the potassium salts appears to be distinctly less than that of the corresponding salts of sodium. Pure solutions of magnesium and calcium salts have no erosive action for a considerable time (six to eight hours), after which the cell membranes begin to be affected.

It is curious to notice that magnesium has little or no stabilising action against the erosive power of potassium, whereas calcium has a well-marked action. It is conceivable that this fact may ultimately be correlated with the observation that calcium and potassium are mutually inhibitory in the case of certain tissues; also, if the cell surface be normally maintained by magnesium, then it is possible that potassium may be able to penetrate under normal conditions whereas sodium does not.

Since we can control the destructive effects of the monovalent salts by means of magnesium and calcium, it is interesting to note that the direct action of the different monovalent ions upon the ciliary mechanism forms a well-marked series.

In the following experiment the monovalent salts were present in the molecular concentration of the sodium in normal sea-water; the divalent salts were in the same concentration as they normally occur in sea-water:—

Solution.	P_H.	Movement of terminal cilia.					
		2′.	5′.	10′.	20′.	30′.	60′.
$LiCl$, $MgCl_2$, $CaCl_2$	7·0	0	0	0	0	0	0
$NaCl$, $MgCl_2$, $CaCl_2$	7·2	+ +	+ +	+ +	+ +	+ +	+ +
NH_4Cl, $MgCl_2$, $CaCl_2$	7·0	+ + ⊕	+ + ⊕	+ + ⊕	+ + ⊕	+ + ⊕	+ + ⊕
KCl, $MgCl_2$, $CaCl_2$	7·2	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Sea-water (HCl)	7·2	+ +	+ +	+ +	+ +	+ +	+ +

0 = no movement.
+ + = normal rate of beat.
+ + ⊕ = quicker than normal.
+ + + = very rapid beat.

Apart, therefore, from their action on the cell-wall, the monovalent ions exert a definite effect on the rate of beat of the cilia. The order in which the various ions increase the rate of beat is well marked, and is as follows:—

$$\mathrm{Li} < \mathrm{Na} < \mathrm{NH_4} < \mathrm{K}.$$

The stoppage of the cilia in the lithium mixture is of the same nature as that observed in acids (Gray (5)), and it is not surprising to find that by raising the alkalinity of the lithium mixture, a rapid and well-maintained beat takes place.

The fact that the presence of potassium induces a more rapid rate of beat than a corresponding amount of sodium enables us to test whether the normal semipermeability of the cell membrane is essential to ciliary activity or not. The experiment can be performed as follows:—Gill fragments are placed in an isotonic solution of sodium citrate until all movement has ceased. Individual pieces are now placed in the following solutions and their behaviour noted.

M/2 NaCl	P_H 7·8	No recovery. Cilia remain healthy, but cells absorb water rapidly.
Sea-water	P_H 7·8	Cilia at once become opaque. Cells rapidly absorb water.
M/2 KCl	P_H 7·8	Marked recovery of beat: recovery is temporary, and cells rapidly swell.

If, whilst the period of recovery in M/2 KCl is well marked, the gills are transferred to sea-water or to any solution containing calcium, the cilia instantly become opaque, and the cells swell rapidly. It is clear, therefore, that although such cilia may beat rapidly in the presence of potassium chloride, yet the semipermeability of the cell-wall has been entirely destroyed by the previous treatment with sodium citrate. The only metal capable of re-stabilising the cell-wall is magnesium.

A further study of this problem obviously leads to a consideration of the nature of the cell-membrane itself, and although our knowledge is very far from complete, interesting analogies may be pointed out in the case of non-living systems. One of the most characteristic features of cell-membranes is their capacity for allowing weak alkalies and acids to pass into the cell and yet exclude the strong alkalies and acids. These facts have, of course, led to the suggestion that the cell-membrane is essentially lipoid in nature—or that, at least, it contains a lipoid phase. Now Clowes (2) has shown that the nature of an oil and water system depends upon the nature of the ions present in the system. The truth of this statement may be very simply verified as follows:—Into five test-tubes are placed 10 c.c. of olive oil containing a little oleic acid, and an equal volume of test solution, together with 0·5 c.c. N/10 NaOH. The tubes are then thoroughly shaken by hand, and examined after about five minutes.

Clowes maintains that a system of water-drops in a continuous phase of oil may be converted into a system of oil-drops in a watery phase by means of sodium chloride. It must be pointed out, however, that grave difficulties attend the suggestion that the surface of the cell possesses a continuous oily phase; nevertheless, these experiments indicate the possible mode of operation of bivalent metals on the cell-surface, if the latter in any way resembles an oily emulsion.

In some ways an even closer analogy to the experiments described in this paper is provided by the experiments of Schryver (10). This author has shown that when a 2 per cent. sodium cholate solution is heated in the presence of calcium a gel is formed. This gel is eroded by solutions of the salts of monovalent cations, but is completely stable when in contact with a solution of balanced monovalent and divalent cations. The erosive power

Table III.

Test tube.	Test solution.	Nature of resulting system.
A	10 c.c. distilled water	A uniform milk-white emulsion of oil drops in water. Discontinuous phase—OIL. Continuous phase—WATER.
B	10 c.c. M/2 NaCl	Two phases separate out in equal volumes— (i) Water almost free of oil. (ii) Oil almost free of water.
C	10 c.c. $M/10\ MgCl_2$	A uniform greasy emulsion of water drops in oil. Discontinuous phase—WATER. Continuous phase—OIL.
D	10 c.c. $M/10\ CaCl_2$	Same as C.
E	10 c.c. sea-water	Two phases separate— (i) A large volume of water drops in a continuous oil phase. (ii) A small volume of oil drops in water.

of the monovalent ions depends upon several factors: (*a*) their concentration, (*b*) the salts existing in the gel. In concentrations equivalent to those used in the present series of experiments (viz., about M/2), the erosive power of LiCl is much less than that of Na or K. A study of Schryver's results shows that the analogy between them and the experiments here described is strong. It remains, however, to be proved that such a system as a cholate gel possesses the same semipermeable properties as a living membrane.

Summary.

(i) The ciliated cells of *Mytilus edulis* are insensitive to the following anions:

$$Cl', NO_3', Br', I', Acet', SO_4'',$$

as long as the normal equilibrium of the cations $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$ and $Mg^{\cdot\cdot}$ is maintained in the surrounding medium.

(ii) In solutions containing tartrates and citrates, the bivalent metals $Mg^{\cdot\cdot}$ and $Ca^{\cdot\cdot}$ are probably not present in the ionic state, and the cells behave as though these metals were absent.

(iii) There is no justification for the statement that the order in which anions affect ciliary motion is the reverse of the order in which they affect muscular movement.

(iv) Pure solutions of sodium salts destroy the normal semipermeable nature of the cell-membrane, and the cell colloids behave as an elastic gel in direct contact with the external medium.

(v) The action of sodium salts can be inhibited by magnesium or by calcium. Probably in normal sea-water the stability of the cell-membrane is due to magnesium and not to calcium.

(vi) In balanced solutions the monovalent cations have a direct effect upon the rate of ciliary movement. The rate of movement in solutions of the same hydrogen ion concentration is slowest in lithium and fastest in potassium. The ions can be arranged in the following well-marked series:—

$$Li^{\cdot}, Na^{\cdot}, NH_4^{\cdot}, K^{\cdot}.$$

(vii) The normal semipermeability of the cell-wall is not an essential condition for ciliary movement.

(viii) The way in which solutions of the different metals affect the cell-surface is in complete agreement with the effect which they produce on the electrical conductivity of the cell.

(ix) There is a marked analogy between the action of salts on the living cell-membrane and on a cholate gel, or oil and water emulsion.

The expenses of this research were in part met by a grant from the Government Grant Committee of the Royal Society.

BIBLIOGRAPHY.

1. H. Bechhold, 'Die Kolloid in Biologie und Medizin,' p. 273 (1912).
2. G. H. Clowes, 'Journal of Phys. Chemistry,' vol. 20, p. 407 (1916).
3. J. Gray, 'Quart. Journal Micro. Science,' vol. 64, p. 345 (1920).
4. J. Gray, 'Phil. Trans. Roy. Soc.,' B, vol. 207, p. 481 (1916).
5. J. Gray, 'Proc. Camb. Phil. Soc.,' vol. 20, p. 352 (1921).
6. R. Höber, 'Physik. Chem. der Zelle und Gewebe,' pp. 508, 532 (1914).
7. R. S. Lillie, 'Amer. Journ. of Phys.,' vol. 17, p. 89 (1906).
8. W. J. Osterhout, 'Science,' vol. 39, p. 544 (1914).
9. T. Sakai, 'Zeit. für Biologie,' vol. 64, p. 1 (1914).
10. J. B. Schryver and M. Hewlett, 'Roy. Soc. Proc.,' B, vol. 89, p. 361 (1916).
11. C. Shearer, 'Journ. of Hygiene,' vol. 18, p. 337 (1919).